PB84-144310

Maternal Hepatic and Embryonic Effects of 1,2,4- Trichlorobenzene in the Rat

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1983

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completir

1. REPORT NO.	2.	3. PB84-144310
EPA-600/J-83-135		

4. TITLE AND SUBTITLE
Maternal Hepatic and Embryonic Effects of 1,2,4-Trichlorobenzene in the Rat (Journal Version)

5. REPORT DATE
1983

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Kirk Kitchin and Marian Ebron

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Experimental Biology Division
Health Effects Research Laboratory
US Environmental Protection Agency
Research Triangle Park, NC 27711

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Office of Research and Development
Health Effects Research Laboratory
US Environmental Protection Agency
Research Triangle Park, NC 27711

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA-600/11

15. SUPPLEMENTARY NOTES
Published In: Environmental Research 31: 362-373, 1983

16. ABSTRACT

The possible maternal hepatic and reproductive effects of 1,2,4-trichlorobenzene (TCB) were assessed in rats given 0, 36, 120, 360, and 1200 mg/kg/day of TCB on Days 9-13 of gestation. The animals were sacrificed on Day 14 of pregnancy. Maternal deaths (2/9 rats 6/6 rats) were recorded in the 360 and 1200 mg/kg/day treatment groups and body weight gain was significantly decreased in the 360 mg/kg/day TCB group. Maternal liver weight liver/body weight ratio, and hepatic microsomal protein content were unaffected by TCB treatment. Although Day 14 NADPH-cytochrome c reductase activity was affected only at 360 mg/kg/day TCB, the maternal hepatic microsomal cytochrome P-450 content was significantly increased by administration of both 120 and 360 mg/kg/day of TCB. Hepatic microsomal aminopyrine N-demethylase, ethoxyresorufin O-deethylase, and UDP-glucuronyl transferase activity towards p-nitrophenol were also increased at 120 and 360 mg/kg/day TCB. Glutathione S-transferase activity to 1-chloro-2,4-dinitrobenzene and 1,2 dichloro-4-nitrobenzene were both increased by pretreatment with TCB. Although pretreatment with 360 mg/kg/day TCB did not increase resorptions, embryolethality, or teratogenicity, embryonic development was significantly retarded by all four growth criteria used (head length, crown-rump length, somite number, and protein content).

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	13
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

ENVIRONMENTAL RESEARCH 31, 362–373 (1983)

EPA-600/J-83-135
JOURNAL ARTICLE

Maternal Hepatic and Embryonic Effects of 1,2,4-Trichlorobenzene in the Rat[1]

KIRK T. KITCHIN[2] AND MARIAN T. EBRON

Experimental Biology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

Received July 21, 1982

The possible maternal hepatic and reproductive effects of 1,2,4-trichlorobenzene (TCB) were assessed in rats given 0, 36, 120, 360, and 1200 mg/kg/day of TCB on Days 9–13 of gestation. The animals were sacrificed on Day 14 of pregnancy. Maternal deaths (2/9 rats, 6/6 rats) were recorded in the 360 and 1200 mg/kg/day treatment groups and body weight gain was significantly decreased in the 360 mg/kg/day TCB group. Maternal liver weight, liver/body weight ratio, and hepatic microsomal protein content were unaffected by TCB treatment. Although Day 14 NADPH-cytochrome *c* reductase activity was affected only at 360 mg/kg/day TCB, the maternal hepatic microsomal cytochrome *P*-450 content was significantly increased by administration of both 120 and 360 mg/kg/day of TCB. Hepatic microsomal aminopyrine *N*-demethylase, ethoxyresorufin *O*-deethylase, and UDP-glucuronyl transferase activity towards *p*-nitrophenol were also increased at 120 and 360 mg/kg TCB. Glutathione *S*-transferase activity to 1-chloro-2,4-dinitrobenzene and 1,2 dichloro-4-nitrobenzene were both increased by pretreatment with TCB. Although pretreatment with 360 mg/kg/day TCB did not increase resorptions, embryolethality, or teratogenicity, embryonic development was significantly retarded by all four growth criteria used (head length, crown–rump length, somite number, and protein content).

INTRODUCTION

1,2,4-Trichlorobenzene is used as a dye carrier, dielectric, solvent, fire retardant, and industrial intermediate (Jones, 1973; Dow Chem. Co., 1975, 1979; Hawley, 1977). It occurs in the environment both from industrial sources and as a breakdown product of other chlorinated organics (Jondorf *et al.*, 1955). Concern about the possible health effects of TCB has risen after TCB was identified in drinking water supplies and waste water (Gaffney, 1976) and in tissues of fresh water fish (Veith *et al.*, 1979).

The number and position of chlorine atoms on the benzene ring influence the lipid solubility, biotransformation, and toxicity of chlorinated benzenes (Allen *et al.*, 1979; Ware and West, 1977; Ariyoshi *et al.*, 1975). The more highly chlorinated benzenes which lack the two adjacent hydrogen atoms required for rapid expoxide formation (Kohli *et al.*, 1976) bioaccumulate in food chains. Chronic toxicity is the primary possible environmental problem with these compounds. In contrast, chlorinated benzenes with two vicinal hydrogens will not bioaccu-

[1] This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

[2] To whom requests for reprints should be addressed.

mulate to as great an extent. However, these molecules pose a greater acute toxicity hazard. Acute toxicity is generally inversely related to the degree of chlorination (Allen *et al.*, 1979). Furthermore, the low molecular weight and absence of charge of all chlorinated benzenes should permit substantial placental transport of these lipophilic compounds.

In this study the maternal hepatic and embryonic effects of administration of varying doses of 1,2,4-trichlorobenzene were determined in rats. TCB was administered on Days 9 to 13 of pregnancy, the period of maximal susceptibility to teratogenesis, and experimental animals were sacrificed on the 14th day. Day 14 embryos exposed to TCB *in vivo* were evaluated for growth and morphological parameters.

METHODS

Experimental animals and dosing. 1,2,4,-Trichlorobenzene (Aldrich Chem. Co., >99% pure) dissolved in corn oil (A&P) was orally administered in doses from 36 to 1200 mg/kg/day to pregnant rats on Days 9 to 13 of gestation in a volume of 2 ml/kg. Controls were orally fed corn oil alone. Time-pregnant Sprague–Dawley rats (CD strain, Charles River Breeding Laboratories, Kingston, Mass.) were given food and water *ad libitum*. The day sperm were detected in the vaginal smear was designated as Day 1 of gestation. Pregnant females were housed two per plastic nestbox cage and six or more rats were used in each TCB dosage group. Standard research laboratory conditions regarding bedding materials, light/dark cycle, temperature, and humidity were maintained throughout the experiment. Animals were killed by decapitation on the morning of Day 14 and their livers were excised and weighed. Hematoxylin and eosin-stained sections of control and treated livers were examined histologically. In some studies, the uteri were removed from 12 pregnant rats in control and 360 mg/kg/day TCB groups and the embryos examined for growth and differentiation parameters (Kitchin *et al.*, 1981). Live embryos were observed with a Wild 7A stereomicroscope and scored as normal or abnormal as compared to corn oil-treated controls. The presence of a beating heart, the somite number, and embryo size were recorded. After homogenization in 50 mM potassium phosphate buffer, pH 7.4, the total embryonic protein content was determined (Lowry, 1951).

Enzyme assays. Five grams of maternal rat liver were homogenized in 20 ml of 0.02 M Tris–HCl buffer (pH 7.5) containing 1.15 M KCl. Washed microsomes were prepared by ultracentrifugation as described by Kitchin and Woods (1978). The high speed supernatant (1 hr 100,000*g*) was used for glutathione *S*-transferase determinations. Cytochrome *P*-450 content was assayed by the method of Omura and Sato (1964) using an Aminco DW-2 spectrophotometer. All enzyme activities were determined at 37°C under conditions of time and protein linearity. NADPH-cytochrome *c* reductase (LaDu *et al.*, 1971), aminopyrine *N*-demethylase (Nash, 1973; Lucier *et al.*, 1971), and ethoxyresorufin *O*-deethylase (Burke and Mayer, 1974) activities were determined by published procedures. The *in vitro* rates of the glucuronidation of *p*-nitrophenol and phenolphthalein were determined by substrate disappearance using 0.2% Triton X-100 activated microsomes (Mulder, 1970). Microsomal protein content was determined by the biuret method (Gornall

et al., 1949). Laboratory chemicals and reagents were obtained from standard commercial sources.

Statistics. Analysis of variance procedures were used to determine statistically significant effects (Goldstein, 1964). Enumerative data were analyzed by the Fishers exact test (Goldstein, 1964). Quantitative data are presented as the mean ± SEM for five to eight independent observations. For statistical purposes, the litter was considered the experimental unit and a P value of 0.05 was considered significant.

RESULTS

All animals that received 1200 mg/kg/day TCB died by the third day of treatment. Administration of 360 mg/kg/day TCB caused some maternal lethality (2/9 rats) and greatly reduced body weight gain from +37 to −17 grams per rat (Fig. 1). Liver weight, liver/body weight ratio and hepatic microsomal protein content were unaffected by TCB treatment. TCB was a strong hepatic enzyme inducer. Hepatic cytochrome *P*-450 content was elevated from 0.44 to 0.79 and 1.36 nmol/mg protein by treatment with 120 and 360 mg/kg/day TCB respectively (Fig. 2). NADPH-cytochrome *c* reductase activity was almost doubled at the higher dose of TCB.

Both hepatic microsomal aminopyrine *N*-demethylase and ethoxyresorufin *O*-deethylase were induced by administration of 120 and 360 mg/kg/day of TCB to pregnant female rats (Fig. 3). At the higher dose the oxidative enzyme activity in microsomes from TCB-treated dams was approximately double that of controls.

The conjugative enzyme UDP-glucuronyltransferase exhibited a heterogeneous response to TCB. *p*-Nitrophenol and phenolphthalein were used as representative "late fetal" and "neonatal" isoenzyme substrates, respectively (Wishart *et al.*, 1978). The isoenzyme "late fetal" UDP-glucuronyltransferase was induced by the medium and high dose of TCB while "neonatal" UDP-glucuronyltransferase was not (Fig. 4).

The second conjugative isoenzyme enzyme system examined was hepatic cytosolic glutathione *S*-transferase. Enzymatic conjugation of 1,2-epoxy-3-(*p*-nitrophenoxy)propane with glutathione was not affected by prior TCB treatment. At 120 mg/kg/day TCB, glutathione *S*-transferase activity for 1-chloro-2,4-dinitrobenzene was increased and at the 360 mg/kg/day TCB dose, glutathione *S*-transferase activity for this substrate as well as for 1,2-dichloro-4-nitrobenzene was increased. Maximal enzyme induction was about threefold above control values for both of these substrates. Hepatic reduced glutathione levels were significantly depressed from 5.05 to 3.19 μmol/g liver by administration of five daily doses of 360 mg/kg/day TCB. In this experiment, about 18 hr elapsed between the last TCB dosing and sacrifice.

Liver histology in the control and 36 mg/kg/day TCB group was unremarkable. At 120 mg/kg/day only one of nine rats showed a slight degree of hepatocellular hypertrophy. Upon histological examination, seven of eight pregnant female rats fed 360 mg/kg/day TCB exhibited moderate hepatocellular hypertrophy. In addition, liver specimens were obtained from three rats which had died after being treated with 1200 mg/kg/day TCB. Although autolysis was evident in two of the

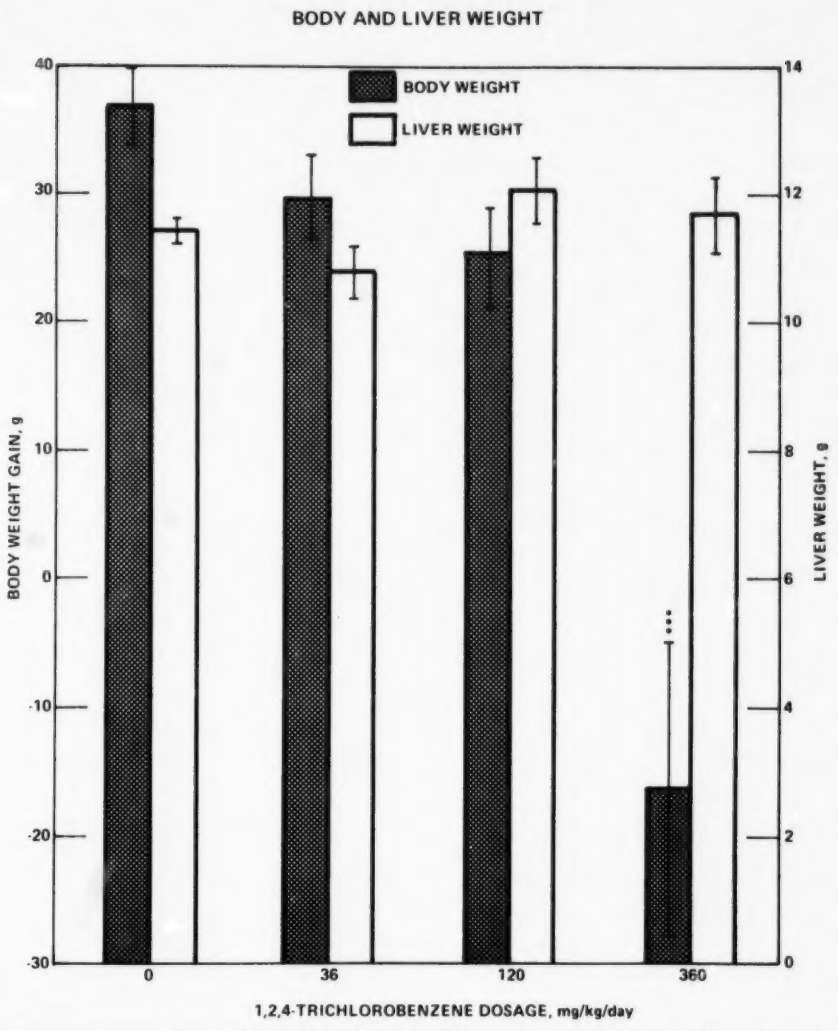

FIG. 1. Pregnant female rats were given the indicated dose (mg/kg/day) of 1,2,4-trichlorobenzene on Days 9–13 of gestation by gavage. The body weight gain (gestational Day 14–Day 8) and Day 14 liver weights are expressed as the mean + SEM for five to eight animals. $***P < 0.001$.

three liver specimens, moderate to moderately severe multifocal necrosis was seen in all rats in this treatment group.

Administration of TCB to pregnant dams adversely affected embryonic growth (Table 1). Resorptions, embryolethality, and abnormalities were not increased to a statistically significant extent by TCB treatment. Embryolethality was confined to 3 of the 12 TCB-treated litters. In the TCB group, the two embryonic anomalies

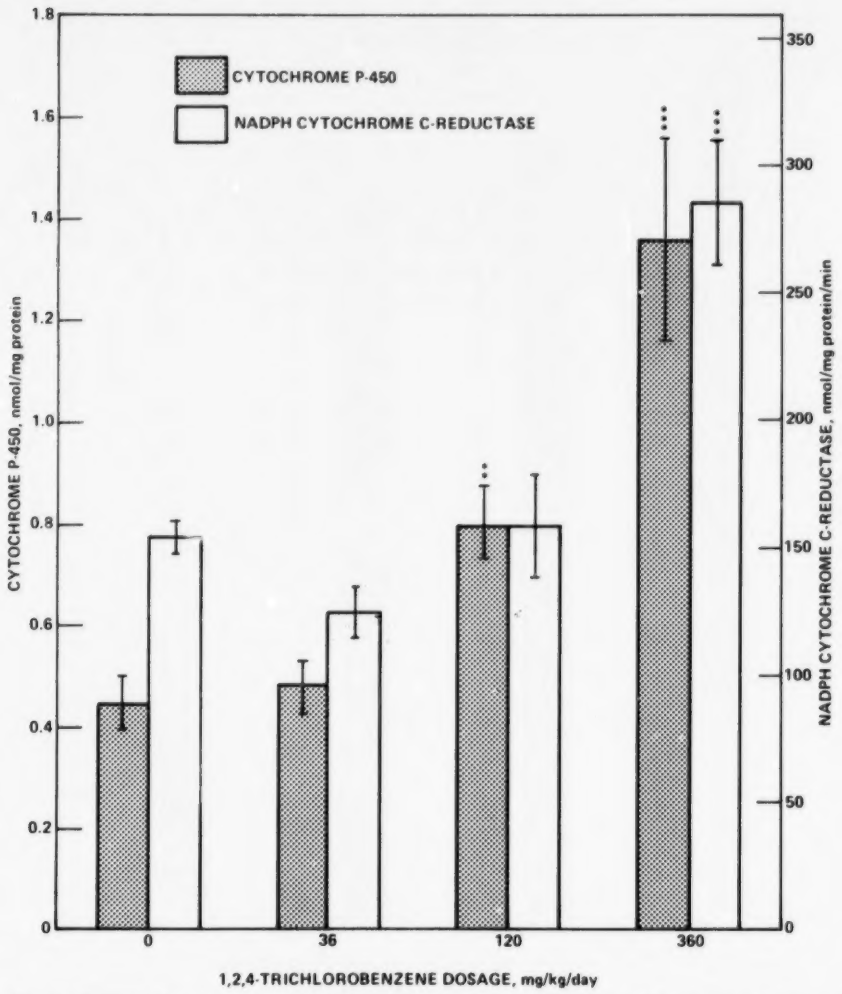

FIG. 2. Pregnant female rats were given 1,2,4-trichlorobenzene on Days 9–13 of gestation by gavage. On Day 14 the animals were sacrificed and the hepatic microsomal cytochrome *P*-450 content and NADPH-cytochrome *c* reductase activities determined. The data are presented as the means ± SEM for five to eight animals. $^{**}P < 0.01$, $^{***}P < 0.001$.

(from two different TCB-treated dams) observed were misshapen backs. Embryonic growth parameters were all significantly lowered by TCB administration to pregnant rats during organogenesis. Although embryonic head length, crown–rump length, and somite number were decreased by TCB administration by only about 5%, the total embryo protein content was reduced by 23%.

MATERNAL HEPATIC ENZYME INDUCTION

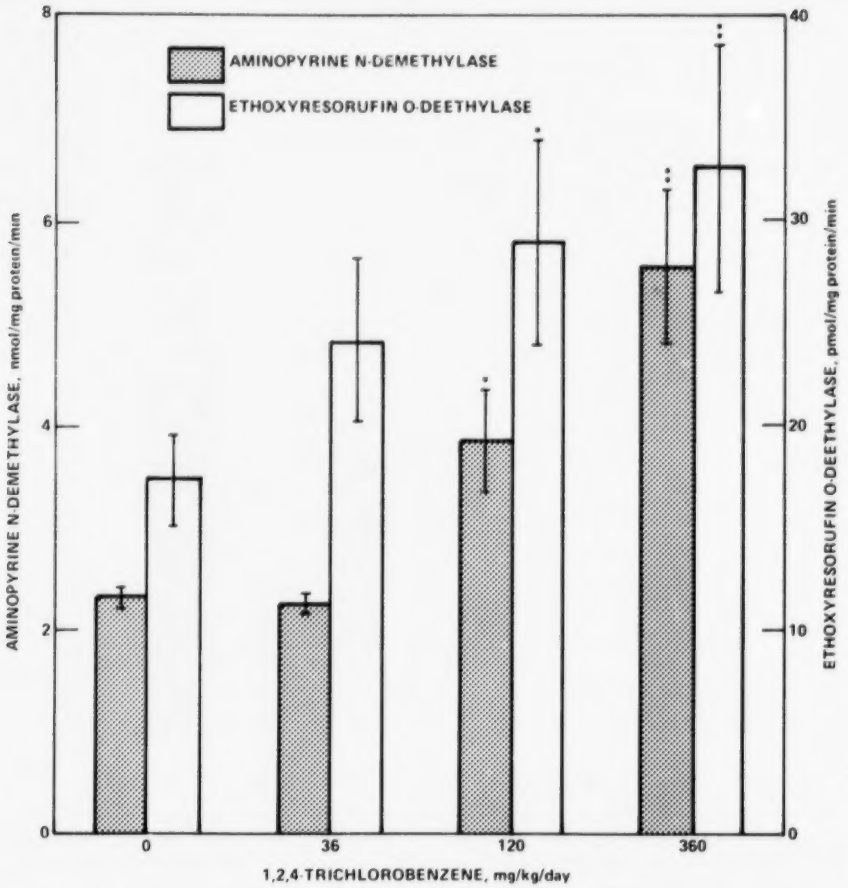

FIG. 3. Day 14 hepatic microsomal aminopyrine *N*-demethylase and ethoxyresorufin *O*-deethylase activities were determined after administration of several different oral doses of 1,2,4-trichlorobenzene to rats on Days 9–13 of gestation. Data are means ± SEM for six to eight animals. $^{*}P < 0.05$, $^{**}P < 0.01$.

DISCUSSION

At 120 mg/kg/day TCB, hepatic enzyme induction of cytochrome *P*-450 levels, aminopyrine *N*-demethylase, ethoxyresorufin *O*-deethylase, UDP-glucuronyl transferase activity towards the "late fetal substrate" *p*-nitrophenol and glutathione *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene were observed.

At 360 mg/kg/day, some maternal lethality and the expected hepatic glutathione content depression (both at 5 and 18 hr after sacrifice) occurred. The full potency

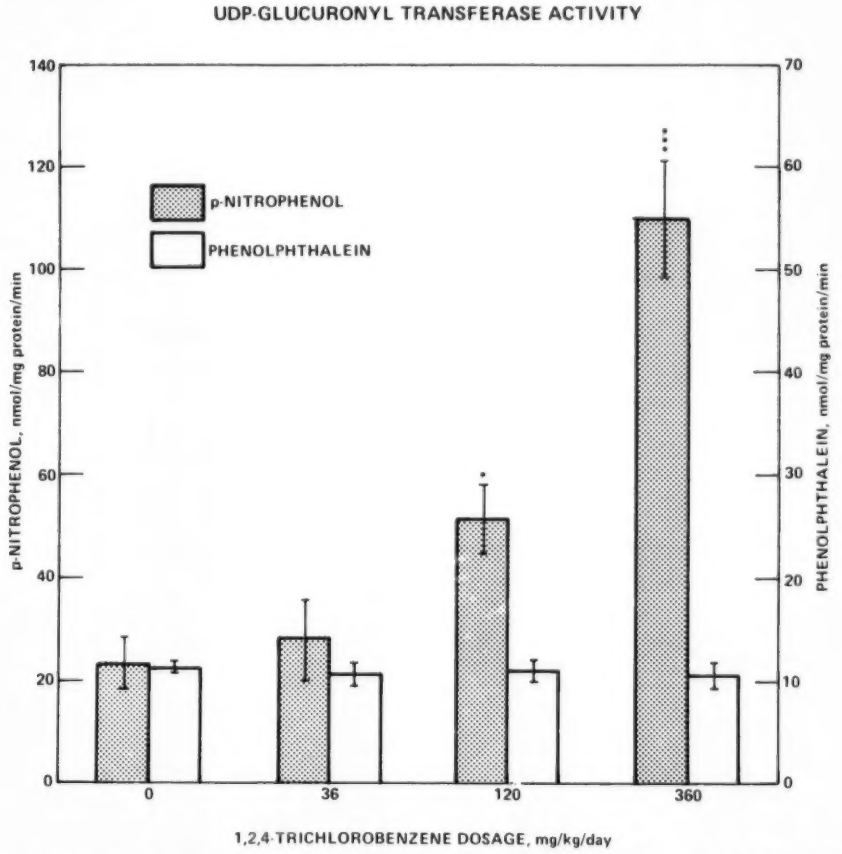

FIG. 4. Pregnant female rats were given various doses of 1,2,4-trichlorobenzene during Days 9–13 of gestation by gavage. On Day 14 the animals were sacrificed and the *in vitro* rates of glucuronidation of *p*-nitrophenol and phenolphthalein determined. Data are means ± SEM for five to eight animals. $^{*}P < 0.05$, $^{***}P < 0.001$.

of TCB as an hepatic enzyme inducer was obvious at this dosage: the activity of seven out of the nine enzyme activities studied were increased. The results of this study can be compared to those in which phenobarbital and β-naphthoflavone were given to pregnant rats in a previous experiment (Kitchin and Ebron, 1983). Compared to phenobarbital and β-naphthoflavone pretreatment, TCB was a stronger enzyme inducer for five and four of the seven responsive enzymes, respectively. Only in respect to induction of ethoxyresorufin *O*-deethylase activity was TCB less potent than β-naphthoflavone. A summary of the direction and magnitude of the effects of TCB on maternal hepatic parameters is shown in Table 2. TCB was not found to be significantly embryolethal or dysmorphogenic during embryonic organogenesis (the Day 9–13 period). Embryonic growth retardation

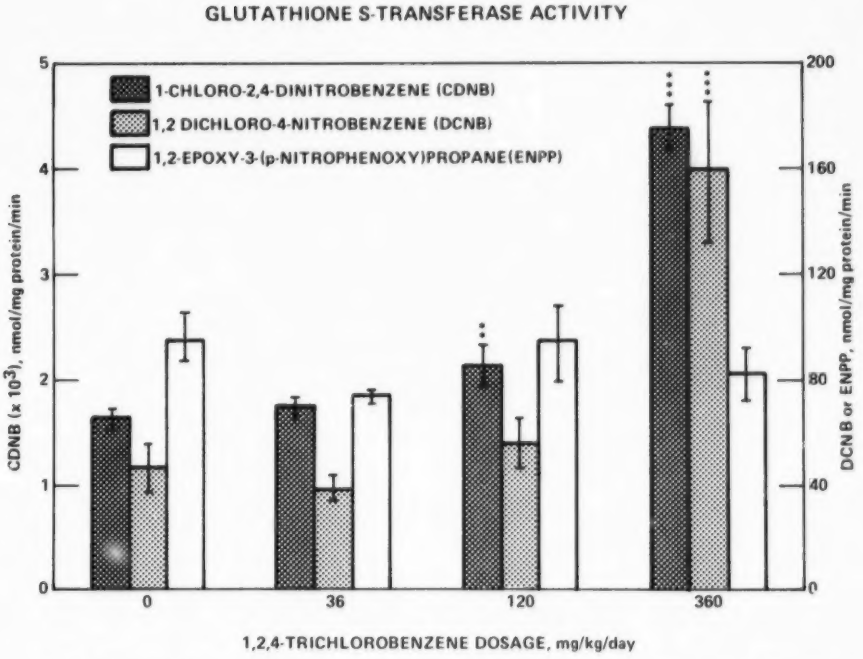

FIG. 5. Pregnant female rats were given the indicated doses of TCB on Days 9–13 of gestation by gavage. On Day 14 the animals were sacrificed and the hepatic high-speed supernatant gluthathione *S*-transferase activity towards CDNB, DCNB, and ENPP determined. Data are means ± SEM for five to eight independent observations. $^{**}P < 0.01$, $^{***}P < 0.001$.

was caused by 360 mg/kg/day of TCB. Embryonic protein content was 23% lower in embryos of the TCB-treated dams. Further reproductive and developmental studies are required to determine if embryos of TCB treated rats can catch up to normal Day 21 fetal size or if the growth retardation is permanent.

Ariyoshi *et al.* (1975) fed 250 mg/kg/day TCB to nonpregnant Wistar rats for 3 days and observed increases in liver weight, hepatic microsomal protein, cytochrome *P*-450, and aminopyrine *N*-demethylase activity. In adult male rats administration of 40 mg/kg/day of TCB for 14 days increased hepatic microsomal cytochrome *P*-450, NADPH-cytochrome *c* reductase, aryl hydrocarbon hydroxylase, and UDP-glucuronyltransferase activity toward the "late fetal" substrate 1-napthol (Carlson and Tardiff, 1976). Their study found TCB induced-hepatic microsomal enzyme induction persisted after a 30-day recovery phase. Both these investigators found TCB to be an extremely potent hepatic enzyme inducer, an observation confirmed in our study. Rats exposed to TCB (25, 100, or 400 ppm) in drinking water did not show decreased fertility, growth, or viability in a multigeneration reproduction study (Robinson *et al.*, 1981). The highest dose for female rats calculated in that study was 53.4 mg/kg/day (Robinson *et al.*, 1981).

TABLE 1

EFFECTS OF 1,2,4-TRICHLOROBENZENE ON EMBRYONIC GROWTH AND MORPHOGENESIS IN THE RAT

		Resorptions		Dead embryos		Embryonic parameters				
	Implantations	Resorption/ implantations	Affected litters/ litters	Affected embryos/ embryos	Affected litters/ litters	Abnormalities	Head length (mm)	Crown–rump length (mm)	Somites	Protein (μg)
Vehicle	14.16 ± 0.31 (12)	9/170	5/12	0/161	0/12	0/26	4.72 ± 0.06 (26)	8.17 ± 0.09 (26)	49.6 ± 0.6 (26)	2670 ± 108 (26)
1,2,4-Trichlorobenzene (360 mg/kg/day)	12.50 ± 0.79 (12)	12/150	7/12	25/138	3/12	2/24	4.43 ± 0.10 (24)	7.69 ± 0.17 (24)	47.7 ± 0.6 (24)	2053 ± 121 (24)
Level of significance	$p < .05$	NS	NS	$p < .001$	NS	NS	$p < .05$	$p < .05$	$p < .05$	$p < .01$

Note. Dams were given daily oral doses of vehicle or 360 mg/kg/day TCB on Days 9–13 of gestation. Twelve vehicle and TCB litters were examined. They were sacrificed by ether administration on the morning of Day 14 and the uteri examined for implantations, resorptions, and embryonic parameters. Two embryos, one from each uterine horn, from each female were used in the determination of various embryonic parameters. Quantitative data are expressed as the means ± SEM for the indicated number of embryonic observations. The litter was used as the experimental unit in statistical comparisons of the five embryonic parameters.

TABLE 2
SUMMARY OF MATERNAL PARAMETERS

								Glutathione S-transferase								UDP-Glucuronyl Transferase	
	Lethality	Body wt	Liver wt	Liver wt/ body wt ratio	Protein/ liver	Protein/ g liver	Gluthathione	CDNB	DCNB	ENPP	NADPH-cytochrome *c*-reductase	Cytochrome *P*-450	Aminopyrine *N*-demethylase	Ethoxyresorufin *O*-deethylase	*p*-Nitrophenol	Phenolphthalein
Corn oil																
36 mg/kg/ day TCB										↓						
120 mg/kg/ day TCB							↑	↑↑				↑↑	↑	↑↑	↑	
360 mg/kg/ day TCB		↓↓↓					↓↓	↑↑↑	↑↑↑		↑↑↑	↑↑↑	↑↑	↑↑	↑↑↑	
1200 mg/kg/ day TCB	↑↑↑															

Note. This figure summarizes the maternal findings. AT 1200 mg/kg/day TCB, all animals died and were not evaluated biochemically. Two possibly spurious findings were an increase in glutathione content 18 hr after dosing with 120 mg/kg TCB and a decrease in glutathione *S*-transferase activity for 1,2 - epoxy - 3 - (*p* - nitrophenoxy)propane (ENPP). These effects were not dose related. The direction of the arrow indicates an increase or decrease from control values. One, two, and three arrows represents $P < 0.05$, 0.01, and 0.001, respectively. The number of experimental animals per group is the same as in Figs. 1–5.

This chronic TCB exposure is less than the 120 and 360 mg/kg/day acute doses used in our study.

To date, the maternal hepatic and embryonic effects of three chlorinated benzenes (1,2,4,5-tetra-, 1,2,3,4-tetra-, and 1,2,4-trichlorobenzene) have been determined (Kitchin, 1982; Kitchin and Ebron, 1983; Kitchin and Ebron, in press). Of these three compounds, 1,2,4-trichlorobenzene is the most potent in respect to maternal toxicity, glutathione depletion, hepatic enzyme induction, and embryonic toxicity. Within the chlorinated benzene series, the presence of two vicinal hydrogens increases the rat maternal hepatic and embryonic toxicity. Thus, to date it appears that the same structure–activity relationships useful in predicting the toxicity of chlorinated benzenes to male experimental animals (Allen *et al.*, 1979; Matthews and Kato, 1979) hold for maternal hepatic and embryonic toxicity in rats.

ACKNOWLEDGMENTS

We thank Ms. Cynthia Drum, Ms. Pam Marsh and Mr. Ellis Allen for their excellent technical assistance. Mmes. Vickie Hawks and Carole Alberts helped prepare this manuscript and Dr. Dave Svensdgaard gave helpful advise on the statistical analysis. Dr. Jim Proctor of Experimental Pathology Laboratories Inc. performed the histological evaluations.

REFERENCES

Allen, J. R., Hargraves, W. A., Hsia, M. T. S., and Lin, F. S. D. (1979). Comparative toxicology of chlorinated compounds on mammalian species. *Pharmacol. Ther.* **7**, 513–547.

Ariyoshi, T. K., Ideguchi, K., Iwasaki, K., and Arakai, M. (1975). Relationship between chemical structure and activity. II. Influences of isomers in dichlorobenzene, trichlorobenzene and tetrachlorobenzene on the activities of drug-metabolizing enzymes. *Chem. Pharm. Bull.* **23**, 824–830.

Burke, M. D., and Mayer, R. T. (1974). Ethoxyresorufin: Direct fluorometric assay of a microsomal *O*-dealkylation which is preferentially inducible by 3-metholocholanthrene. *Drug Metab. Disposit.* **2**, 583–588.

Carlson, G. P., and Tardiff, R. G. (1976). Effect of chlorinated benzenes on the metabolism of foreign organic compounds. *Toxicol. Appl. Pharmacol.* **36**, 383–394.

Dow Chemical Company (1975). "Organic Chemicals from Dow-chlorinated Aromatics." Dow Chemical Company, Midland, Mich.

Dow Chemical Company (1979). "Comments on the Third Report of the TSCA Interagency Testing Committee: Chlorinated Benzenes, Tri, Tetra, and Penta-." Dow Chemical Company, Midland, Mich.

Gaffney, P. E. (1976). Carpet and rug industry case study I: Water and waste water treatment plant operation. *J. Water Pollut. Control Fed.* **48**, 2590–2598.

Goldstein, A. (1964). "Biostatistics," pp. 63–93. MacMillan, New York.

Gornall, A. G., Bardawill, C. J., and David, M. M. (1949). Determination of serum proteins by means of the briuret reaction. *J. Biol. Chem.* **177**, 751–766.

Hawley, G., ed. (1977). "The Condensed Chemical Dictionary," 9th ed., p. 886. Van Nostrand Reinhold, New York.

Jondorf, W. R., Park, D. V., and Williams, R. T. (1955). Studies in detoxification: Metabolism of halogenobenzenes 1,2,3-, 1,2,4-, and 1,3,5-trichlorobenzenes. *Biochem. J.* **61**, 512–521.

Jones, H. R. (1973). "Pollution control in the textile industry," pp. 8–9. Noyes Data Corp., Park Ridge, New Jersey.

Kitchin, K. T. and Ebron, M. T. (1983). Maternal hepatic and embryonic effects of 1,2,3,4-Tetrachlorobenzene in the rat. *Toxicology*, 1983.

Kitchin, K. T. and Ebron, M. T. Maternal hepatic effects of 1,2,4,5-tetrachlorobenzene in the rat. *Environ. Res.*, in press.

Kitchin, K. T., Schmid, B. P., and Sanyal, M. K. (1981). Teratogenicity of cyclophosphamide in a coupled microsomal activating/embryo culture system. *Biochem. Pharmacol.* **30**, 59–64.

Kitchin, K. T., and Woods, J. S. (1978). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) induction of aryl hydrocarbon hydroxylase in female rat liver. Evidence for de novo synthesis of cytochrome *P*-448. *Mol. Pharmacol.* **14,** 890–899.

Kitchin, K. T. (1982). Hepatic effects of 1,2,4,5-tetrachlorobenzene in the pregnant rat. *Toxicologist* **2,** 506.

Kohli, J., Jones, D., and Safe, S. (1976). The metabolism of higher chlorinated benzene isomers. *Canad. J. Biochem.* **54,** 203–208.

LaDu, B. N., Mandel, H. G., and Way, E. L. (1971). "Fundamentals of Drug Metabolism and Disposition," pp. 576–577. Williams & Wilkins, Baltimore.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurements with Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

Lucier, G., McDaniel, O., Brubaker, P., and Klein, R. (1971). Effects of methylmercury hydroxide on rat liver microsomal enzymes. *Chem.–Biol. Interact.* **4,** 265–280.

Matthews, H. B., and Kato, S. (1979). The metabolism and disposition of halogenated anomatics. *Ann. N.Y. Acad. Sci.* **320,** 131–136.

Mulder, G. J. (1970). The effect of phenobarbital on the submicrosomal distribution of uridine diphosphate glucuronyltransferase from rat liver. *Biochem. J.* **117,** 319–324.

Nash, T. (1953). The colormetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.* **55,** 416–421.

Omura, T., and Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.* **239,** 2370–2378.

Robinson, K. S., Kavlock, R. J., Chernoff, N. and Gray, L. E. (1981). Multigeneration study of 1,2,4-trichlorobenzene in rats. *J. Toxicol. Environ. Health* **8,** 489–500.

Veith, G. D., Kuehl, D. W., Leonard, E. N., Publishi, F. A., and Lemke, A. F. (1979). Polychlorinated biphenyls and other organic chemical residues in fish from major watersheds of the U.S. *Pestic. Monit. J.* **13,** 1–11.

Ware, S. R., and West, W. L. (1977). "Investigation of selected potential environmental contaminants: Halogenated benzenes final report." Office of Toxic Substances, Environmental Protection Agency, Washington, D.C.

Wishart, G. J., Campbell, M. T., and Dutton, G. J. (1978). Functional heterogeneity of UDP-glucuronyltransferase. *In* "Conjugation Reactions in Drug Biotransformation" (A. Aitio, ed.), pp. 179–187. Elsevier/North-Holland Biomedical Press, Amsterdam.